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Expression of transient receptor potential vanilloid receptor 1 and toll-like receptor 4 in aggressive periodontitis and in chronic periodontitis

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Background and Objective: The objective of the present study was to evaluate the expression and the distribution of the transient receptor potential vanilloid receptor 1 (TRPV1) and of toll-like receptor 4 (TLR4) in tissue samples from patients with periodontal disease (aggressive periodontitis and chronic periodontitis) and from healthy controls.

Material and Methods: Ten tissue samples from each disease group (aggressive periodontitis and chronic periodontitis) and from healthy subjects were obtained during routine oral surgical procedures. Subgingival specimens were collected from sites with advanced loss of support (probing depth > 5 mm) and specimens from the corresponding healthy controls were obtained during tooth extraction for orthodontic reasons or following surgical extraction of an impacted third molar. The distribution of TRPV1 and TLR4 receptors in human gingival tissue was studied by immunohistochemistry.

Results: Both TLR4 and TRPV1 were detected in gingival tissues from healthy subjects, and from patients with chronic periodontitis and aggressive periodontitis, particularly in gingival keratinocytes, fibroblasts, inflammatory cells and the endothelial lining of capillaries in connective tissues. Histologic examination of the samples from healthy controls disclosed that clinically healthy gingiva does not correspond to histologically healthy gingiva. Subsequently, these samples were redesignated as gingivitis samples. TRPV1 was down-regulated in all cell types in samples obtained from patients with chronic periodontitis compared to samples obtained from patients with gingivitis, whereas TLR4 was down-regulated only in the epithelium and in gingival fibroblasts. In contrast, the levels of these markers in patients with aggressive periodontitis were similar to those in healthy patients.

Conclusion: Local expression of TRPV1 and TLR4 in gingival tissues may contribute to both physiological and pathological processes in the periodontium. Our data suggest that TRPV1 and TLR4 may play a role specifically in the pathophysiology of chronic periodontitis.

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Clinical and experimental evidence supports the notion of crosstalk between our nervous and immune systems (1,2). The involvement of the sensory nervous system in generating some of the manifestations of inflammation was first demonstrated by Bayliss (3) more than 100 years ago. He reported that vasodilatation followed cutaneous neural stimulation, suggesting that in addition to their sensory function, these neurons have an efferent neurosecretory role.

Stimulation of the peripheral sensory nerves evokes not only vasodilatation but also other characteristic signs of inflammation (4). On activation, specific sensory neurons release neuropeptides from their terminals. These peptides, in turn, act on immune cells and on vascular smooth muscle (5), resulting in vasodilatation (mainly calcitonin gene-related peptide associated), protein extravasation (mainly of substance P) and hypersensitivity. This phenomenon has been termed 'neurogenic inflammation' (4). This neurogenic inflammation is mediated mainly by small sensory neurons that are sensitive to capsaicin. Capsaicin has been used as a topical treatment for a variety of pain syndromes for many centuries (6). Its receptor [transient receptor potential vanilloid receptor 1 (TRPV1), formerly known as VR1] has recently been cloned (7). This receptor is widely distributed throughout the peripheral nervous system (8), in sensory nerve cell bodies, in nerve terminals supplying peripheral target organs and in central afferent terminals in the spinal cord. It also is expressed outside the nervous system in epithelial cells, in tissues such as gut, skin, lung and bladder, as well as in immune cells such as mast cells and Langerhan's cells (9).

Evidence for a role of neurogenic inflammation in periodontal disease was first found in the late 1980s. In rodents, an increased density of sensory nerves was demonstrated in periapical abscesses, and these innervations were intensified particularly at sites of severe periodontal inflammation and necrosis (10). Further studies showed that electrical stimulation of branches of the trigeminal nerve induced neurogenic inflammation in the periodontal and oral tissues (11). Additionally, capsaicin-sensitive sensory nerves supplying the junctional epithelium were demonstrated to be involved in neurogenic plasma extravasation in the rat gingiva, and this was prevented by sensory denervation (12,13) or by prior exposure to capsaicin (13). In humans, topical application of capsaicin to healthy gingiva temporarily induced local elevations in the concentration and in the activation of MMP-8 (a major destructive protease active in periodontal disease) in the gingival crevicular fluid (14).

Toll-like receptor (TLR) 4, a member of the TLR superfamily, is the principal pattern recognition receptor on innate immune cells. The TLRs are involved in the recognition of microbial structures, such as the lipopolysaccharide (LPS) from *Porphyromonas gingivalis*, a significant periodontopathogen (15,16). They provide a prompt response and protection against microbial challenge. The expression of *TLR4* was demonstrated in gingival epithelial cells (17,18), human gingival fibroblasts (19) and monocytes (20). The recognition of microbial components by TLRs results in the release of proinflammatory cytokines that are necessary to activate potent immune responses (21). Qureshi *et al*. (22) demonstrated that *TLR4*-deficient mice are hyporesponsive to LPS, supporting a role of these proteins in LPS recognition. Furthermore, exposure to LPS induces a reduced response to subsequent LPS challenge, a phenomenon known as LPS hyporesponsiveness (23). Thus, the expression profile of TLR4 in chronic periodontal disease has received much attention in the literature. However, the distribution of TLR4 in aggressive periodontitis (AgP) has not yet been reported. Interestingly, TLR4 was shown to be expressed on peripheral sensory nerve fibers in inflamed pulp tissues (24), suggesting that bacterial products such as LPS can directly activate these neurons through TLR4, and these results could be elaborated further to show the possible link of TLR4 with the activity of neurons.

Although the responses, to capsaicin, of sensory neurons in the oral tissues have been studied for some time, the cellular distribution of the receptor thought to be responsible for triggering this response has not yet been investigated. We hypothesized that alteration in the expression of the capsaicin receptor, TRPV1, in periodontal disease-affected gingival tissues can contribute to disease development and that this alteration could be mediated through the variation in expression of TLR4. In order to test this hypothesis, we analyzed the expression and the distribution of TRPV1 in the gingiva of periodontally healthy subjects as well as in periodontal pockets from patients with chronic periodontitis (CP) and AgP. Furthermore, in an attempt to establish whether the two receptors have similar distribution profiles, we investigated the expression of TLR4 in periodontal tissues.

Material and methods

Sample collection

Gingival tissue samples were obtained from 10 patients with CP, 10 patients with AgP and 10 periodontally healthy subjects, all of whom provided informed consent. The study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from the local Ethics Committee of Ondokuz Mayıs University. The healthy samples were harvested from teeth extracted for orthodontic reasons or were obtained from patients who had undergone surgical extraction of impacted third molars. The sample sites met the following criteria: probing depth not exceeding 3 mm; absence of bleeding; and no radiographic evidence of alveolar bone loss. However, histologic examination of the healthy samples revealed that the clinically healthy gingiva did not correspond to a histologically healthy gingiva. Previous studies have demonstrated that apparently clinically healthy gingivae manifest histological evidence of inflammation coinciding with that seen in marginal gingivitis. Subsequently, these samples were redesignated as gingivitis samples (25,26). Periodontally involved tissues, characterized by advanced loss of support (probing depth < 5 mm), were

obtained from patients undergoing periodontal surgery by internal beveled incisions. CP and AgP were diagnosed, according to the 1999 American Academy of Periodontology classification (27), by measuring probing depth, clinical attachment loss and bleeding on probing, and by examining radiographs. Patients with CP had periodontal pockets > 5 mm and clinical attachment loss > 4 mm in at least four nonadjacent teeth. Patients were diagnosed as having AgP when presenting with the following: an interproximal probing pocket depth and clinical attachment loss of ≥ 5 mm; radiographic bone loss of ≥ 30% of the root length on at least two permanent teeth, of which at least one was a first molar or an incisor; under 30 years of age; and the amounts of microbial deposits were inconsistent with the severity of periodontal tissue destruction. Patients who had a systemic disease that affects the periodontium and patients who were smokers were excluded.

Immunohistochemistry

The gingival biopsies were immediately fixed in 4% paraformaldehyde and then processed into paraffin blocks. Serial paraffin sections were cut into thicknesses of 4–6 μm and mounted on slides. The slides were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked for 10 min with 3% hydrogen peroxide in distilled water. For antigen retrieval, the slides were heated in a microwave for 10 min in 0.01 M citrate buffer solution (pH 6.0) and treated with Protein Block Serum-Free (Dako, Carpinteria, CA, USA) for 5 min at room temperature. The slides were then incubated with primary antibody to TRPV1 (sc-20813; 1:200 dilution) and to TLR4 (sc-10741; 1:200 dilution) (both from Santa Cruz Biotechnology, Santa Cruz, CA, USA) at room temperature for 60 min, and then with secondary antibody for 30 min. Finally, the sections were then reacted in 3,3′-diaminobenzidine, counterstained with Mayer's hematoxylin and mounted. As negative controls, sections were treated with phosphate-buffered saline or with normal rabbit immunoglobulin G (Santa Cruz Biotechnology), instead of with primary antibodies, in the presence of all other steps.

Assessment of immunohistochemical staining

The slides were analyzed under a light microscope by pathologists who had no knowledge of the clinical diagnosis. The intensity and extent of staining was determined for whole tissue sections. The cell types were identified based on histomorphologic features. Immunohistochemical staining of each slide was evaluated according to the extent and the intensity of staining. The extent of staining was the percentage of stained cells and was scored semiquantitatively using a scale of 0–4, as follows: 0, no expression; 1+, 1–25% of cells stained; 2+, 26–50% of cells stained; 3+, 51–75% of cells stained; and 4+, 76–100% of cells stained. The intensity of staining was scored as 1 for weak staining (faint, light yellow), as 2 for moderate staining (brown) and as 3 for strong staining (dark brown). Additionally, an immunoreactive score was calculated by multiplication of the percentage of positive cells with the staining intensity, as proposed by Krajewska *et al*. (28). For example, when 50% of the cells in a specimen showed a moderate staining intensity, a composite score of 4 was given ($2 \times 2 = 4$).

Statistical analysis

All statistical analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Expression scores of TLR4 and TRPV1 in the samples from patients with periodontal diseases and from control subjects were compared using the Kruskal–Wallis test. Two-group comparisons were assessed using the Mann–Whitney *U*-test. Statistical significance was set at $p < 0.05$.

Results

The expression of TLR4 and TRPV1 was detected immunohistochemically in all tissues affected with gingivitis and periodontitis (CP and AgP). Both TRPV1 and TLR4 localized in gingival keratinocytes, fibroblasts, inflammatory cells and the endothelial lining of capillaries in the connective tissues (Figs 1 and 2). The extent and the intensity of labeling were studied separately in each cell type.

TRPV1

The percentage of TRPV1-positive cells was generally lower in tissues from patients with CP than in tissues from patients with gingivitis, while there was no difference between tissues from patients with AgP and tissues from patients with gingivitis (Fig. 1). The extent of staining score was 1 in 90% (9/10) of the samples of epithelial cells in CP, whereas it was 1 in only 30% of the samples of epithelial cells in gingivitis, 2 in 10% and 3 in 60%. The extend score was 1 in 40% (4/10) of the samples of epithelial cells in AgP and 3 in 60%. The percentages of TRPV1-positive fibroblasts, endothelial cells and inflammatory cells were also lower in CP. The mean extent of staining score was significantly different in CP and gingivitis for all cell types, whereas the difference between AgP and gingivitis samples was not statistically significant in any of the cell types (Table 1).

In addition to the percentage of immunopositive cells, TRPV1 immunointensity was evaluated. In gingivitis, 70% of specimens contained fibroblasts with moderate or strong TRPV1 immunostaining, whereas in CP, only 10% of specimens contained fibroblasts with moderate or strong TRPV1 immunostaining. This difference was statistically significant ($p = 0.008$). On the other hand, TRPV1 immunointensity was not significantly different among epithelial ($p = 0.06$), endothelial ($p = 0.52$) or inflammatory ($p = 0.098$) cells in CP and gingivitis (Table 1). When TRPV1 immunointensity was evaluated in samples obtained from patients with AgP or gingivitis, the difference between the groups was not statistically significant across the cell types (Table 1). The composite scores for TRPV1 were lower, in general, in all cell types in samples from patients with CP than in samples from patients with gingivitis, whereas the difference between the composite scores for TRPV1 was not statistically

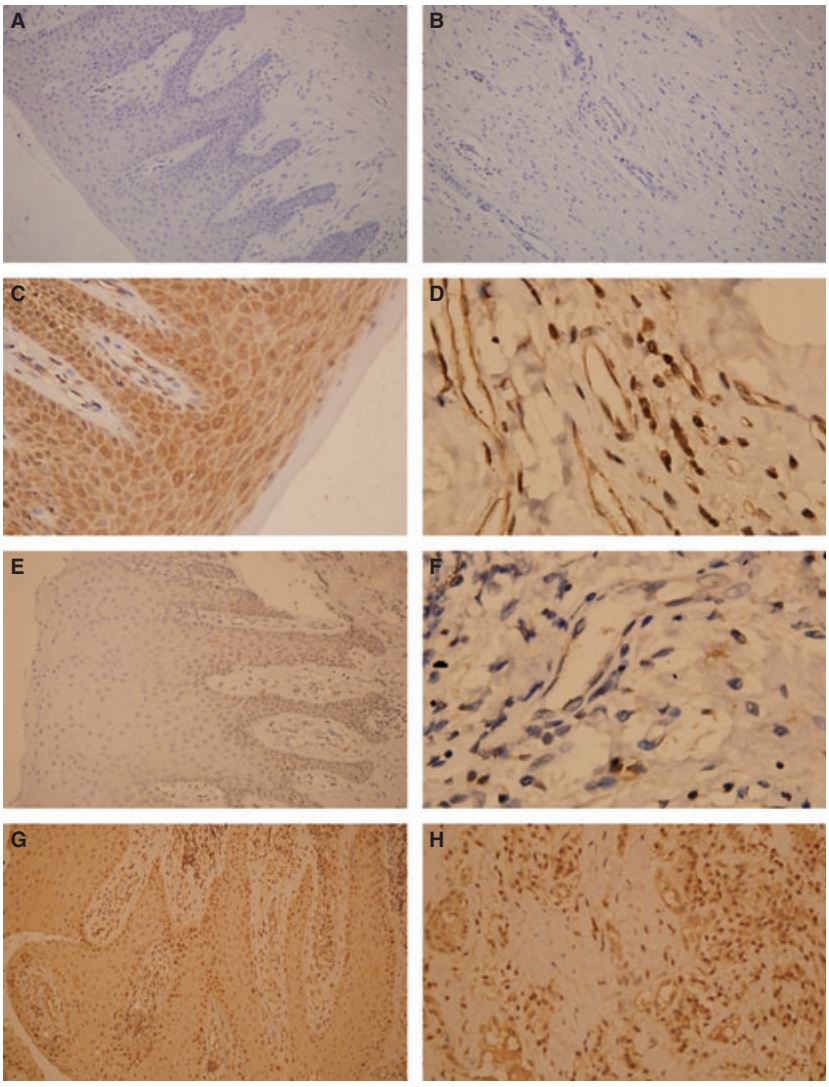

Fig. 1. Examples of cytoplasmatic transient receptor potential vanilloid receptor 1 (TRPV1) immunoreactivity (brown reaction product). (A) and (B) Negative controls. (C) Immunolocalization of TRPV1 in the epithelial cell layers of gingival tissues from control subjects. Note the high levels of strongly expressed TRPV1 in keratinocytes [Diaminobenzidine (DAB) chromogen, ×200]. (D) TRPV1 is strongly expressed in the connective tissue of control specimens (DAB chromogen, ×1000). (E) Weak TRPV1 immunoreactivity in keratinocytes from patients with chronic periodontitis (CP). Note that TRPV1 was sparsely stained in the keratinocytes (DAB chromogen, ×400). (F) Sparse and weak TRPV1 immunoreactivity in connective tissue from patients with CP. Note that the intensity of TRPV1 was strong in endothelial cells but it was sparsely distributed (DAB chromogen, ×1000). (G) Strong immunostaining for TRPV1 in both epithelium and connective tissue (H) of gingival tissue samples from patients with aggressive periodontitis (AgP).

significant for any cell type in samples from patients with AgP or in samples from patients with gingivitis (Table 1, Fig. 1). Finally, when the total intensity and expression of TRPV1 were evaluated, patients with CP exhibited less frequent and less intense expression of TRPV1. However, the intensity of expression did not reach statistical significance ($p = 0.085$). By contrast, when combined scores were evaluated, the difference between the groups was statistically significant (Table 1).

TLR4

Similarly to TRPV1, the percentage of TLR4-positive cells was lower in epithelial cells ($p = 0.003$) and in fibroblasts ($p = 0.002$) from CP patients compared with gingivitis patients. However, unlike TRPV1, we did not find any difference among endothelial ($p = 0.06$) or inflammatory ($p = 0.15$) cells (Table 2). On the other hand, the extent of TLR-4 immunostaining in AgP and gingivitis subjects was not statistically significant for any cell type ($p = 0.94$; Table 2). The TLR4 immunointensity was decreased in epithelial cells in CP specimens compared with gingivitis specimens ($p = 0001$); however, the difference in TLR4 immunointensity between the other cell types in CP and gingivitis specimens was not statistically significant. Likewise, the difference in TLR4 immunointensity between AgP specimens and gingivitis specimens was not statistically significant in any of the cell types (Table 2). The average composite score for TLR4 was significantly lower in epithelial cells ($p = 0.001$) and fibroblasts ($p = 0.01$) in CP patients than in gingivitis subjects. The difference in endothelial ($p = 0.15$) or inflammatory ($p = 0.28$) cells was not statistically significant. On the other hand, similarly to the extent of staining and intensity of staining scores for TLR-4, the average composite score between AgP and gingivitis was not statistically significant. Lastly, the total intensity and the expression of TLR4 were evaluated. Patients with CP exhibited less frequent ($p = 00.1$) and less intense expression of TLR4 ($p = 0.009$). Similarly, comparison of combined scores for all cells revealed a significant difference between CP and healthy controls ($p = 0.003$, Table 2).

Discussion

In the present study, TRPV1 was found in specimens from patients with different forms of periodontitis and in control subjects. In the gingival tissues, several types of cell expressed TRPV1, in particular, keratinocytes, fibroblasts, endothelial cells and inflammatory cells, which is a finding not yet reported for humans. Furthermore, we demonstrated that although both TRPV1 and TLR4 receptors were down-regulated in subjects with CP

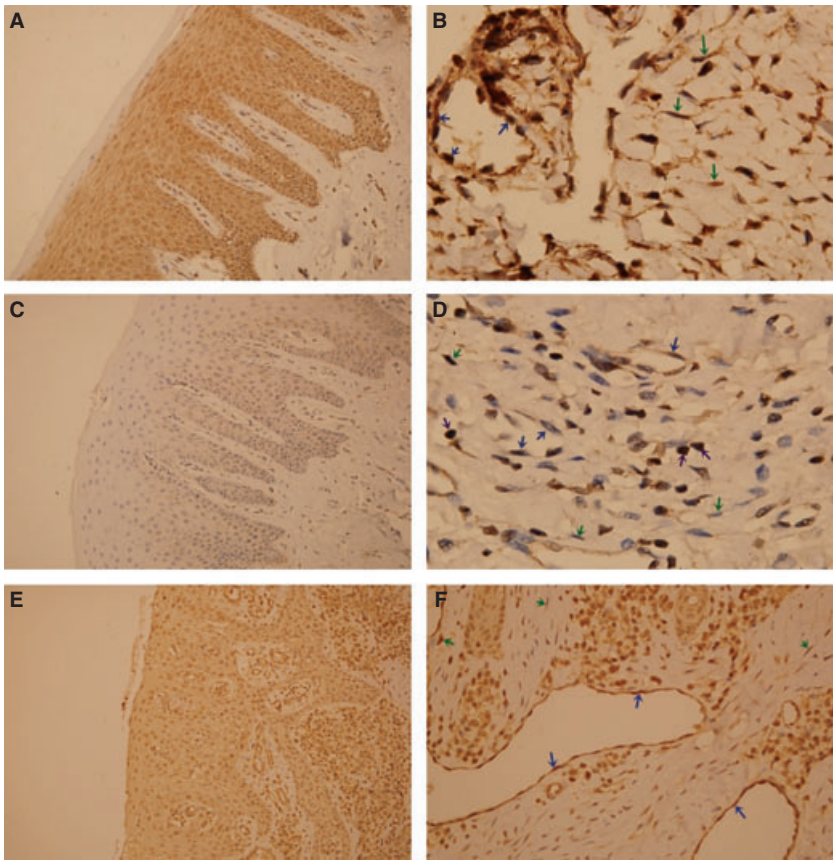

Fig. 2. Examples of cytoplasmatic toll-like receptor 4 (TLR4) immunoreactivity (brown reaction product). (A) Immunolocalization of TLR4 in the epithelial cell layers of gingival tissues from control subjects. Note the high levels of strongly expressed TLR4 in keratinocytes (DAB chromogen, ×200). (B) Strong expression of TLR4 in endothelial cells (blue arrows) and in fibroblasts (green arrows) of control subjects [Diaminobenzidine (DAB) chromogen, ×1000]. (C) Weak TLR4 immunoreactivity in keratinocytes in samples from patients with chronic periodontitis (CP) (DAB chromogen, ×200). (D) Sparse, but strong, expression of TLR4 in the connective tissue of samples from patients with CP. Inflammatory cells (purple arrows), endothelial cells (blue arrows) and fibroblasts (green arrows) are indicated (DAB chromogen, ×1000). (E) Similarly to control subjects, strong immunostaining for TLR4 was found in both the epithelium and the connective tissue (F) of gingival tissue samples from patients with aggressive periodontitis (AgP). Endothelial cells (blue arrows) and fibroblasts (green arrows) are indicated.

compared to subjects with gingivitis, there was no statistically significant difference in the distribution and intensity of these receptors between subjects with AgP and subjects with gingivitis in all cell types. Our study is the first to report the distribution of the TLR4 also in AgP.

The TRPV1 protein is a nonselective cation channel expressed by sensory nerves. It is activated when exposed to noxious heat (> 42°C), acid (protons) or the pungent ingredient of chilli peppers, capsaicin. It can also be activated by the proinflammatory peptide bradykinin, by anandamide and by endogenous hydrogen ions (29,30). Tissue damage associated with inflammation or infection produces protons and an array of lipid-derived second messengers, such as anandamide, that activate or sensitize nociceptor terminals at the site of injury (31). Indeed, TRPV1 seems to be directly involved in peripheral neurogenic inflammation (9).

Capsaicin, the classic TRPV1 agonist, has been widely used to investigate the biology of sensory neurons and neurogenic inflammation. Topical application of capsaicin results in a cascade of inflammatory events, such as erythema, and in the release of proinflammatory mediators in the skin and mucosa (9). A neurogenic component appears to be involved in many inflammatory diseases, including periodontitis (32). Avellan *et al.* (14) applied capsaicin topically to periodontally healthy volunteers and observed a significant local elevation in the amount of MMP-8, a major destructive protease in periodontal disease, in the gingival crevicular fluid of the adjacent teeth. This elevation and activation lasted for several minutes. These results suggest that TRPV1 has a role in the pathogenesis of periodontal diseases. Wadachi & Hargreaves (24) showed that TLR4 receptors were co-localized with TRPV1 on sensory nerves in inflamed pulp tissue and trigeminal nerves. These results suggest that bacterial products, such as LPS, may directly activate these neurons through TLR4 receptors. Interestingly, in our study, both TRPV1 and TLR4 were down-regulated in CP compared with gingivitis. In addition to sensory neurons, TRPV1 is expressed by non-neuronal cells, such as mast cells and keratinocytes (33–35). Thus, TRPV1 may be involved in the neuronal–non-neuronal cellular network in the periodontium. Moreover, the presence of VR1 receptors on epithelial cells implies a role in monitoring the oral environment or perceiving pain (24). The molecular and biological mechanism behind the down-regulation of TRPV1 receptors in CP needs to be clarified. It is possible that prolonged and repeated exposure to LPS, indirectly through TLR4 receptors or through other TRPV1 ligands produced during inflammation, desensitizes both TLR4 and TRPV1 receptors similarly to the phenomenon known as 'capsaicin desensitization' (9). Subsequent down-regulation of these receptors may have a role in sustaining the chronic nature of the disease, and the ability to accomplish tolerance to bacterial challenge may represent a difference between CP and AgP.

It was interesting to note that unlike tissues from patients with CP, the expression of TRPV1 and TLR4 in

Table 1. Mean staining extent, intensity and combined scores of transient receptor potential vanilloid receptor 1 (TRPV1) in aggressive periodontitis (AgP), chronic periodontitis (CP) and gingivitis groups

	Extent scores	*p*-value	Intensity scores	*p*-value	Combined scores	*p*-value
Epithelium						
CP	1.1 ± 0.10	0.014*	1.30 ± 0.21	0.056*	1.40 ± 1.13	0.017*
Gingivitis	2.1 ± 0.31		2.10 ± 0.31		5.30 ± 1.27	
AgP	2.2 ± 0.33	0.80**	1.60 ± 0.31	0.25**	4.00 ± 0.55	0.540**
Endothelium						
CP	1.20 ± 0.20	0.03*	2.0 ± 0.33	0.52*	2.60 ± 0.78	0.039*
Gingivitis	2.50 ± 0.27		2.3 ± 0.30		6.40 ± 1.15	
AgP	2.20 ± 0.33	0.53**	1.6 ± 0.31	0.12**	3.80 ± 0.55	0.200**
Fibroblasts						
CP	1.00 ± 0.00	< 0.0001*	1.20 ± 0.20	0.008*	1.20 ± 0.20	0.001*
Gingivitis	2.63 ± 0.26		2.37 ± 0.32		6.75 ± 1.21	
AgP	2.20 ± 0.33	0.39**	1.60 ± 0.31	0.10**	3.80 ± 0.55	0.140**
Inflammatory cells						
CP	1.00 ± 0.00	0.001*	1.40 ± 0.27	0.098*	1.40 ± 0.27	0.010*
Gingivitis	2.38 ± 0.32		2.13 ± 0.35		5.75 ± 1.35	
AgP	2.20 ± 0.33	0.76**	1.60 ± 0.31	0.25**	4.00 ± 0.55	0.400**
Total						
CP	1.08 ± 0.00	0.002*	1.48 ± 0.27	0.085*	1.63 ± 0.27	0.021*
Gingivitis	2.35 ± 0.27		2.20 ± 0.29		5.83 ± 1.14	
AgP	2.20 ± 0.33	0.93**	1.60 ± 0.31	0.15**	4.00 ± 1.13	0.250**

**p*-value for CP vs. control.
***p*-value for AgP vs. control.

Table 2. Mean staining extent, intensity and combined scores of toll-like receptor 4 (TLR4) in aggressive periodontitis (AgP), chronic periodontitis (CP) and gingivitis groups

	Extent scores	*p*-value	Intensity scores	*p*-value	Combined scores	*p*-value
Epithelium						
CP	1.5 ± 0.27	0.003*	1.10 ± 0.1	< 0.0001*	1.80 ± 0.51	0.001*
Gingivitis	2.8 ± 0.20		2.70 ± 0.21		7.90 ± 0.82	
AgP	2.9 ± 0.10	0.94**	2.10 ± 0.23	0.08**	6.20 ± 0.76	0.090**
Endothelium						
CP	2.0 ± 0.33	0.06*	2.60 ± 0.27	0.91*	5.60 ± 1.15	0.150*
Gingivitis	2.8 ± 0.20		2.70 ± 0.21		7.90 ± 0.82	
AgP	2.9 ± 0.10	0.94**	2.10 ± 0.23	0.08**	6.20 ± 0.76	0.090**
Fibroblasts						
CP	1.4 ± 0.27	0.002*	2.30 ± 0.30	0.3*	3.50 ± 0.96	0.010*
Gingivitis	2.8 ± 0.20		2.70 ± 0.21		7.90 ± 0.82	
AgP	2.9 ± 0.10	0.94**	2.10 ± 0.23	0.08**	6.20 ± 0.76	0.090**
Inflammatory cells						
CP	2.3 ± 0.30	0.15*	2.30 ± 0.30	0.30*	6.10 ± 1.22	0.280*
Gingivitis	2.8 ± 0.20		2.70 ± 0.21		7.90 ± 0.82	
AgP	2.9 ± 0.10	0.94**	2.10 ± 0.23	0.08**	6.20 ± 0.76	0.090**
Total						
CP	1.8 ± 0.22	0.001*	2.08 ± 0.18	0.009*	4.00 ± 0.71	0.003*
Gingivitis	2.8 ± 0.20		2.70 ± 0.21		7.90 ± 0.82	
AgP	2.9 ± 0.10	0.94**	2.10 ± 0.23	0.080**	6.20 ± 0.76	0.090**

**p*-value for CP vs. control.
***p*-value for AgP vs. control.

gingival tissues from subjects with AgP was similar to that in the control tissues. The reason for this observation is unclear, but it is possible that a different mechanism might be responsible for the pathogenesis of these two clinically distinct forms of periodontal diseases. Chronic and aggressive forms of periodontal disease are considered to be different diseases as a result of the differences in clinical features, such as: age of onset; rate of progression; pattern of destruction; signs of inflammation; and relative amounts of plaque and calculus (36). Furthermore, the clinical absence of gingival lesions in AgP compared with CP suggests that AgP may not follow the same sequelae of initiation and progression of disease as in CP (37) The results observed in our study reflect differences in the role

of TRPV1 receptors in the pathogenesis of AgP and CP. Different combinations of pathogenic mechanisms may play a role in the tissue destruction seen in both distinct diseases. Furthermore, it has been proposed that differences in microbial environmental factors may play a role by modifying the host response to the etiologic agent (38).

The expression profiles of TLR4 have been studied extensively in CP. Our findings are in accordance with these studies, showing that TLR4 is down-regulated in patients with CP. Indeed, using the quantitative real-time PCR, a nine-fold reduction in TLR4 expression has been reported in patients with CP compared with healthy persons (39). More recently, Beklen *et al.* (40) confirmed that the expression of TLR4 was lower in diseased tissue than in healthy tissue. As a matter of fact, down-regulation of TLR4 expression is one of the mechanisms responsible for LPS tolerance (23). Wang *et al.* (41) demonstrated that the expression of TLR4 in gingival fibroblasts was attenuated in response to repeated challenge with LPS from *P. gingivalis*. Yet another mechanism responsible for the down-regulation of TLR4 was presented. Certain periodontal pathogens, such as *P. gingivalis* and *Prevotella intermedia*, reduce LPS responsiveness by releasing proteinases that cleave TLR4 receptors (42,43). However, other molecular mechanisms of LPS tolerance may exist and this area requires further attention.

In conclusion, TRPV1 was detected in both the epithelial and the connective tissues of gingiva in patients with AgP, CP and gingivitis. Additionally, we demonstrated that TRPV1, along with TLR4, was down-regulated in CP compared with gingivitis and thus may have a role in the pathogenesis of CP. However, further studies using not only immunohistochemical techniques but also other biologic methods are required to elucidate the possible contributions of TRPV1 to periodontal diseases.

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